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Neurobiology of Aging 23 (2002) 747–769

NEUROBIOLOGY OF AGING

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# Qualitative versus quantitative caloric intake: are they equivalent paths to successful aging?

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Received 21 February 2002; received in revised form 16 April 2002; accepted 22 April 2002

**Abstract**

With the increasing demands placed on our society to perform better for longer, in addition to the large increase in the old segment of our population, a race has begun to forestall or reverse the ubiquitous declines that emerge from growing old. Currently, little is known about the mechanisms responsible for the neuronal degeneration seen during both normal aging and neurodegenerative disease; however, among the prime candidates responsible for producing these effects are free radicals. It has been hypothesized that brain aging results from a progressive inability to cope with such insults as oxidative stress and inflammation. As a result, this inability provides a fertile environment for the subsequent development of neurodegenerative disease. Therefore, if the preservation of neuronal function and associated cognitive and motor performance during aging will enhance the probability of aging successfully, then it is of crucial importance to find ways to preserve or decrease the responsiveness of the brain to these insults. The purpose of this review is to discuss two strategies, caloric restriction and antioxidant supplementation (through foods and supplements), both proven to be successful at protecting the brain from age-related oxidative insults. The two interventions will be compared and contrasted in terms of their effectiveness, safety, and generalization capacity for human treatment.


*Keywords:* Caloric restriction; Antioxidants; Dietary supplementation; Phytonutrients; Aging; Alzheimer's disease; Oxidative stress; Parkinson's disease

## 1. Introduction

The coming of age of the baby boom generation as well as the current social demands to perform better and longer, have begun the race to forestall or reverse the ubiquitous declines that emerge from growing old. Of particular interest are the diseases that increase in frequency as a function of age. These range from cancer to nervous system-related illnesses like Alzheimer's (AD) or Parkinson's disease (PD). By the year 2050, 30% of the total population will be over 65 years of age, and it is likely that the majority will show the correspondent motor and cognitive changes that are associated with old age. Currently, little is known about the mechanisms responsible for the neuronal degeneration seen during both normal aging and neurodegenerative disease; however, among the prime candidates responsible for producing these effects are free radicals.

The free radical hypothesis postulates that both physical and neuronal degeneration seen during normal aging [173] or in diseases such as AD [56] and PD [90] may be due to the increased vulnerability to metabolic and extra-metabolic sources of reactive oxygen species (ROS). As our protective antioxidant system becomes less efficient [76,206] these species can react and damage critical biological molecules in the brain, such as proteins [102], cell membrane lipids [230], and nucleic acids such as DNA and RNA [211]. For example, ROS has been shown to increase the rate of apoptotic cell death and therefore, shift the cell death/proliferation ratio of different CNS cell types [111]. It has been speculated that this increase in apoptotic cell death may be due to DNA fragmentation produced by free radical insults [6,136], since both during aging and in certain neurodegenerative pathologies (AD and PD), the levels of cellular protein oxidation, DNA damage and mutations are increased [211].

Interestingly, in this regard studies have shown that in certain areas of the brain, where there is a close association among the levels of oxidants, such as dopamine, the amount of DNA deletion and the amount of free radical damage [32], there are also functional losses. For example, oxotremorine enhancement of striatal dopamine release ($K^+$ERDA), a measure of muscarinic receptor sensitivity, has been shown to significantly decline with age [94,96,99]. Similar declines have also been shown when striatal slices are exposed to hydrogen peroxide, a potent ROS-generating agent [99].

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0197-4580/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0197-4580(02)00079-9

More importantly, however, is the fact that this damage has been able to be reversed via antioxidant treatment [42]. In vitro treatment of cerebellar granule neurons with the free radical scavenger melatonin has been shown to limit apoptotic cell death induced by the intracellular withdrawal of $K^+$ [210]. Cell culture studies have also demonstrated a strong association between oxidative stresses, decreased levels of glutathione, a potent free radical scavenger, and cell death. Moreover, Taglialatela et al. [228] exposed 2-day-old rats to an atmosphere of 95% oxygen and treated them with buthionine sulfoximine (BSO), a glutathione inhibitor. The brains of the subjects that were exposed to both hyperoxia and limited antioxidant protection showed increased levels of brain apoptosis when compared to controls.

Endogenous antioxidants are also present in the developing brain, and their levels have been shown to decrease in concentration during aging. For example, both ascorbic acid and glutathione exist in high intracellular concentrations in the fetal rat brain [191]. Moreover, cultures of fetal mesencephalic cells treated with ascorbic acid for 7–14 days showed marked increases in glial proliferation and increased neurite growth. Nevertheless, as mentioned earlier, research indicates that the endogenous antioxidant levels in senescent rats are reduced in areas such as the hippocampus, cortex and striatum [259], and human studies support these findings. Overall, the plasma concentration of antioxidant enzymes has been shown to be reduced in both normal old humans and subjects affected by neurodegenerative disease [70,217]. In addition, neuronal studies performed on tissue of AD patients have shown increased OS and damage to proteins such as tau and neurofilaments [215] and A-β-mediated OS-induced cell apoptosis [212].

One other factor that is important here is that oxidative stress is a rather “Gemini twin” of inflammation, and there is a considerable amount of evidence to suggest that even as the brain shows increased vulnerability to oxidative stress in aging, it also shows enhanced sensitivity to inflammation. To this end, Manev and Uz [143] showed increased sensitivity of old rats to kainate-induced excitotoxic brain injuries and enhanced 5-lipoxygenase expression in limbic structures. Such increases in inflammatory reactions involve the subsequent expression of such inflammatory mediators as cytokines, complement proteins, and adhesion molecules. These inflammatory mediators, in turn, may enhance OS and initiate putative detrimental neuronal glial interactions that result in the loss of neuronal function [196,205,223,224,245]. More importantly, just as has been seen with respect to OS, these inflammatory mediators may become further enhanced in neurodegenerative diseases such as AD [194,196]. For example, gliosis is a feature common to virtually every neurodegenerative disease (e.g. multiple sclerosis, AD or prion disease [54,156]).

Thus, it may be that the aged brain, through its inability to cope with such insults as oxidative stress and inflammation, provides a fertile environment for the subsequent development of neurodegenerative disease. In this regard, recent research indicates that oxidative stress in one of the earliest alterations in AD [167] and serves as one, of several, initiators of the disease [263]. Therefore, if the preservation of neuronal function and associated cognitive and motor performance well into old age will enhance the probability of successful aging, then it would be of extreme importance to find ways to preserve or decrease the responsiveness of the brain to these insults. The purpose of this review is to discuss two strategies that have been employed that may have this effect. One of these, involving caloric restriction (CR), has been around for many years and appears to exert its positive benefits by enhancing endogenous protection against insults such as oxidative stress. The other is much less studied but is directed toward achieving the same ends by increasing the dietary intake of antioxidants through foods and antioxidant supplementation.

The present article will critically review and contrast the pros and cons of these two strategies in order to not only evaluate their effectiveness, but also to contrast their safety and generalization capacity for human treatment.

## 2. Caloric restriction

Research suggests that CR decreases sensitivity to oxidative stress by: (a) lowering the metabolic rate with subsequent reductions in ROS generation and (b) increasing the capacity of the body to face these insults by exposure to mild stress [148].

There are a wide variety of methods by which CR has been implemented, all with some degree of success. Methodologies range from alternate day feeding, in which the animals are fed 100% every other day, to daily feeding using caloric-reduction (40–60% of regular diet). Similarly, CR regimens vary in length of treatment, ranging from short-term protocols (3 months) to life-long restriction, where the animals are placed on a CR diet shortly after weaning or during the first few months of life and kept on it until they reach senescence (Table 1).

Striking evidence of the beneficial effects of food restriction comes from the fact that it extends life span in animals such as mice [19,209,218], rats [45,84,106] and primates [198]. For example, Laroque et al. [120] examined the effects of ad lib (AL) feeding and moderate dietary restriction Sprague–Dawley rats and found that the 2-year survival rate was 80 and 74% in food-restricted males and females, respectively, and 28 and 38% in AL-fed males and females, respectively. Moreover, the authors found no correlation between initial body weight and 2 years survival in either group of rats, suggesting that the important factor may be total adult food intake and not initial body weight.

Kim and Choi [110] evaluated the effect of dietary restriction on life span and oxidative stress of SAMP8 mice (a model of dementia) and found that median and maximal life spans were significantly increased (28.5 and 16.4%,

Table 1
Methodologies used in CR studies

| Author | Publication | Start feeding | Duration feeding | Method CR | Conclusion |
|---|---|---|---|---|---|
| Acute CR studies | | | | | |
| Pedersen et al. | Brain Res. 833, 117–220 | 6-week-old mice | Until ALS appeared 236 days | Alternate day feeding | No effect on ALS |
| Zhu et al. | Brain Res. 842, 224–229 | 6-week-old mice | 3 months | Alternate day feeding | Diet benefits ps-1 OS damage in hippocampal slices |
| Duan et al. | J. Neurochem. 76, 619–626 | 2-month-old mice | 3 months | Alternate day feeding | Increased BDNF expression and decreased vulnerability to kianate damage |
| Duan et al. | J. Mol. Neurosci. 16, 1–12 | 3-month-old rats | 3 months | Alternate day feeding | Increased BDNF expression and decreased seizure damage |
| Ehrenfried et al. | Ann. Surg. 223, 592–599 | 22–26-month old | 8 weeks | 60% caloric intake of AL rats | HSP-70 protein and RNA increases in stomach and duodenum |
| Fang Yu et al. | J. Neurosci. Res. 57, 830–839 | 1-month-old rats | 3 months | Alternate day feeding | Ameliorates Ischemia |
| Guo et al. | J. Neurochem. 75, 314–320 | 3-month-old rats | 3 months | Alternate day feeding | Cortical synaptosomes protected from OS by increasing glucose and glutamate uptake |
| Lee et al. | Exp. Neurol. 166, 435–441 | 3-month-old rats | 3 months | Alternate day feeding | apoE |
| Lee et al. | J. Neurosci. Res. 57, 48–61 | 2-DG protocol | | | Produces similar effects to those of CR |
| Lee et al. | J. Mol. Neurosci. 15, 99–108 | 3-month-old rats | 3 months | Alternate day feeding | Increased survival of new hippocampal cells and BDNF expression |
| Aging studies | | | | | |
| Dubey et al. | Arch. Biochem. Biophys. | 3–6-month-old rats | 12–17 months/reversal 1–6 weeks | 60% caloric intake of AL rats | Decreases age-related increments in OS |
| Diao et al. | Brain Res. 763, 276–280 | 4-month-old rats | 20–24 months | Diet with 40% of caloric content of AL diet | Increases evoked DA overflow |
| Dorner et al. | Brain Res. 726, 198–206 | 3- and 10-month-old rats | 18 months | 3 days CR/4 AL for 16-month then 2 days CR/5 AL for 2 months then AL | Increases already high levels of fibronectin in brains of old rats |
| Eckles-Smith et al. | Mol. Brain Res. 78, 154–162 | After weaning | 22–29 months or 3–6 months | NIA caloric restricted mice | Prevents age-related LTP and NMDA receptor subunit NR1deficits |
| Gabbita et al. | Free Radic. Biol. Med. 23, 191–201 | 4-month-old mice | 2–21 months | NIA caloric restricted mice | Attenuates age-related membrane fluidity but does not limit oxy-radical production |
| Major et al. | Neurobiol. Aging 18, 523–526 | 6-week-old rats | 24 months | 15 g per day (50% of AL intake) | Attenuates GFAP mRNA expression in aged rats |
| Melov et al. | Nucl. Acids Res. 25, 974–982 | After weaning | Until 32 months of age | No information | Decreases the number of age-related mtDNA species (Southern blot) |
| Morgan et al. | Neuroscience 89, 687–699 | 3-month-old rats | 21 months | NIA caloric restricted mice | Decreases glial hyperactivity in aged rats |
| Prapurna et al. | Mech. Aging Dev. 92, 133–142 | 6-month-old/ 22-month-old rats | 22 months and 8 weeks | 50% cal intake of AL rats | DNA polymerase increase is dependent on onset and length of CR |

Feeding beginning time, duration of regimen and results acquired using acute and chronic protocols.

respectively) by CR. Similarly, the authors used graded scores in order to quantify the degree of senescence in the subjects and found that CR improved these scores two-fold when compared to AL-fed animals. Moreover, oxidative stress measurements illustrated that both superoxide and lipofuscin levels were significantly lowered at 12 months in the CR mice as compared to control-fed animals. Similar findings were seen with respect to superoxide levels at 4 and 8 months as well, indicating that CR may be effective in reducing free radical levels.

Numerous studies have also demonstrated the capacity of CR to modulate various oxidative stress parameters in the brain. These studies range from oxidative stress attenuation by CR on the levels of DNA damage [220], oxidative stress markers [57,219] and iron deposition [29,31], to increasing the levels of antioxidant enzymes [43,246].

As mentioned earlier, free radical species can react and damage critical biological molecules, including nucleic acids such as DNA and RNA [211], and current research suggests that these insults can be attenuated by food restriction. For example, Sohal et al. [219] explored the role of molecular oxidative damage and caloric intake in the aging process by comparing a product of DNA oxidation (8-OHdG) in five different tissues of mice (skeletal muscle, brain, heart, liver and kidney) as a function of age and in response to dietary restriction. In this study, the authors found that CR mice kept on 60% caloric intake as compared to the AL-fed mice showed a lower concentration of 8-OHdG in all the tissues. Moreover, they also found that amelioration of DNA oxidative damage in the CR rats was greater in the post-mitotic tissues as compared to those undergoing slow mitoses.

In conjunction with this study the same group [220] examined the response of oxidative damage, aging and life span to variations in caloric intake. Results illustrated that CR subjects exhibited a 43% extension in average life span and a 61% prolongation in mortality rate doubling time when compared to AL-fed animals. Similarly, they found that the levels of various oxidative stress markers such as protein carbonyl content, mitochondrial superoxide, and hydrogen peroxide in the brain, heart, and kidney of mice (9, 17, and 23 months) were significantly greater in the AL-fed group when compared to the CR group. Nevertheless, the authors failed to find a clear-cut pattern of age-related or dietary-related changes in the levels of various antioxidant defenses (superoxide dismutase, catalase and glutathione peroxidase).

In a similar study Xia et al. [246] determined the changes in the activities of various antioxidant enzymes (Cu, Zn superoxide dismutase, catalase and glutathione peroxidase) in liver, brain cortex, heart, kidney and intestinal mucosa obtained from 6-, 16- and 26-month-old male Fischer 344 rats placed under life-long CR or fed AL. Contradicting Sohal's results, they found that CR increased the activity of one or more of the antioxidant enzymes in the liver, brain cortex, heart and kidney of the rats and reduced lipid peroxidation, which is usually found to increase with age.

Also found in aged brain and neurodegenerated tissue is the accumulation of iron, a pro-oxidant transition metal [213]. In this regard, Choi et al. [29] measured iron content and ferritin levels in the synaptosomes isolated from the frontal cortices of aged male Fischer 344 rats fed AL or under a food restriction regimen. Comparison of lipid peroxide content and peroxidizability of synaptosomal fatty acids between CR and AL-fed groups demonstrated that CR rats showed more effective suppression of free radical production in the brain synaptosomes. Moreover, lipid peroxide and accumulations of total iron and ferritin in brain synaptosomes of the CR group were also shown to be significantly lower than those of AL-fed rats.

Cook and Yu [31] determined total iron content and oxidative stress levels, as measured by lipid peroxidation, on the kidney, liver and brain of male Fischer 344 rats. CR or AL-fed rats were sacrificed at 6, 12 and 24 months of age. Replicating Choi's results, the authors found that CR suppressed age-related increases in total iron content and lipid peroxidation levels in the brain but not the other organs.

Furthermore, Sohal et al. [221] measured the concentrations of bleomycin-chelatable iron and non-heme iron in various tissues and different regions of the brain of mice fed AL or a calorically restricted (to 60% of AL) diet at different ages. With regard to CR, the authors found that CR had no effect on age-increased iron levels in the brain or heart, but caused a marked increase in the concentration of both bleomycin-chelatable and non-heme iron in the liver and the kidney, thus contradicting previous reports of beneficial attenuation of iron levels by CR.

### 2.1. Glucocorticoids

The above findings suggest that CR may be promoting its beneficial effects by enhancing the capacity of the brain to withstand the insults from inflammation and oxidative stress. However, paradoxically, CR has been shown to mildly increase the levels of circulating corticosteroids [148].

Lee et al. [126] examined the effects of food restriction on the expression of the glucocorticoid receptors (GR) in various areas of the brain and found that the expression of this receptor was significantly decreased in food-restricted subjects, possibly via glucocorticoid-mediated-feedback suppression. More importantly, however, is the fact that GR have been shown to negatively regulate the expression of several genes including those encoding heat-shock protein-70 (HSP-70), which acts as a neuroprotective agent against oxidative stress in the brain [258]. Thus, it may be that the downregulation of GR in the presence of mild stress leads to increasing levels of these proteins, which then may protect the brain from oxidative stress injuries.

In this respect, studies have indicated an increased expression of (HSP-70) as well as glucose-related protein-78 (GRP-78) in synaptosomal preparations obtained from CR rats [72].

### 2.2. Neurotrophins

Similarly, CR has also been shown to increase the levels of other neuroprotective agents such as neurotrophic factors. While the exact role of these factors in the mature nervous system is still unclear, it is known that brain derived neurotrophin factor (BDNF) and other factors are positively regulated by neural activity such as by glutamate [128] and acetylcholine release [112], or negatively regulated by GABA [256,257], thus suggesting that BDNF and other neurotrophic factors may play a role in neuronal signaling. Moreover, BDNF is highly expressed in the hippocampus, a region with high levels of plasticity and that has been shown to play a role in the induction of long-term potentiation [114,179].

Duan et al. [41] have reported that levels of BDNF are significantly increased in the hippocampus, cerebral cortex, and striatum of CR as compared to AL-fed rats. Moreover, they also found that CR significantly reduced the seizure-induced damage to hippocampal neurons, and this effect was diminished by intraventricular administration of a BDNF-blocking antibody, suggesting that manipulation of diet can affect expression of a neurotrophic factor and that BDNF may play a central role in the neuroprotective effects of CR.

There is also evidence that by increasing BDNF levels there are also associated increases in neurogenesis in the dentate gyrus of CR animals assessed via bromodeoxyuridine [126]. These increases in neurogenesis appeared to result from decreased death of newly produced cells, rather than from increased cell proliferation, and illustrate that CR can affect neurogenesis by increasing the survival rates of newly generated neurons via the release of BDNF.

### 2.3. Caloric mimetics

From the findings discussed above, it appears that CR can extend life span in rodents and has been reported to increase the resistance of neurons in the brain to excitotoxic and metabolic insults. Given these beneficial effects, it is reasonable to assume that CR might forestall or at least slow down some of the deleterious effects of aging. However, it is also clear that the employment of CR in humans may be a difficult task, therefore, recent research has focused upon investigating the use of caloric mimetics that reduce glucose availability to cells. Indeed, Lee et al. [125] administered 2-deoxy-D-glucose (2-DG), a non-analog of glucose, to adult rats. This manipulation resulted in a highly significant reduction in seizure-induced spatial memory deficits and hippocampal neuron loss. Moreover, when rat hippocampal cell cultures were pretreated with glutamate and $Fe^{2+}$, 2-DG decreased the vulnerability of neurons to excitotoxic and oxidative insults. Finally, 2-DG treatment increased levels of the stress-responsive proteins GRP-78 and HSP-70 in hippocampal neurons, without affecting levels of the apoptotic markers Bcl-2 or GRP-75, suggesting that mild reductions in glucose availability can increase neuronal resistance to oxidative and metabolic insults via the induction of stress proteins.

Similarly, Yu and Mattson [255] examined the impact of CR and 2-deoxyglucose on ischemic brain injury and found that maintenance of adult rats on a CR regimen resulted in reduced brain damage and improved behavioral outcome in a middle cerebral artery occlusion–reperfusion model (MCAO-R). Moreover, they also found that administration of 2-DG to rats fed AL reduced ischemic brain damage and improved behavioral outcome following MCAO-R. Similarly, CR and 2-DG administration resulted in an increase in the level of the stress protein HSP-70 in striatal cells in vivo, and 2-DG treatment induced HSP-70 in cultured neurons, again suggesting involvement of a preconditioning stress response in the neuroprotective actions of CR and 2-DG.

Given the cited research, it is plausible that CR may be exerting its effects, at least partially, via the reduction of glucose that is available in the brain and concurrent energy/metabolism changes that come with this process [148]. Nevertheless, various sources have contradicted this hypothesis. Based on this hypothesis, if CR was to exert its effects by reducing the levels of glucose availability, metabolic rate and mass would also show general reductions. However, various investigators have found metabolic rate levels to be the same in calorically restricted and AL-fed rodents, hence, concluding that the Rate of Living Theory does not help explain the longer life span of the calorically restricted animal [46,147,150,241].

For example, McCarter and Palmer [150] measured daily metabolic rate under usual living conditions over the life span of barrier-maintained Fischer 344 rats and of animals that were food-restricted. Metabolic rate was measured indirectly by analysis of gas entering and leaving standard rodent cages over a 24-h period. The author found that both groups exhibited similar variations of metabolic rate per unit lean mass over the life span, with metabolic rate decreasing from 6 to 18 months and then increasing from 18 to 24 months of age. Thus, it would appear that the life-prolonging action of CR is not a consequence of reduced metabolic rate per unit metabolic mass, but rather that restricted rats were able to sustain appropriate fluxes of nutrients and appropriate metabolic rate under conditions of fuel utilization, which promote maintenance of cellular homeostasis.

Nevertheless, it is the contention of Greenberg and Boozer [67] that previous accounts of reduced metabolic rates in these animals may not have been reliably calculated. In their study, the authors investigated the reliability of 10 different estimates of metabolic mass (MM) in 21-month-old male Fischer 344 rats fed three different diets to yield a wide range of body compositions. The authors found that the combined weight of the heart, liver, kidneys and brain (OW), ranked using strong correlation with daily caloric intake (DCI) and zero *Y*-intercept on the regression curve of DCI versus the MM, was found to be the best estimate of MM. Moreover, data analyses of the differences in metabolic rate in the three

groups of rats showed that the significance of these differences depended on the estimate of MM used. Therefore, because previous investigators used other methods rather than a more reliable estimate such as OW, their finding that CR and AL-fed animals show no difference in metabolic rate may have been premature.

### 2.4. Neurodegenerative diseases and CR

Currently various models of neurodegenerative diseases such as AD and PD have been developed in order to elucidate the mechanisms of action and find a cure for these diseases [40,44,62]. Recent reports suggest that CR may be at least partially effective in retarding or reversing some of the changes seen in these animal models. For example, mice transgenic for presenilin-1 (PS-1) mutations show increased responsiveness to excitotoxic injury [264], which is also seen during normal aging. However, CR has been shown to reduce the sensitivity of hippocampal CA1 and CA3 neurons in CR mice when compared to animals fed AL. Moreover, the levels of the lipid peroxidation following the excitotoxic insult have been found to be lower in CR/PS-1 mice compared to mice fed AL, again suggesting that suppression of oxidative stress may be one mechanism underlying the neuroprotective effect of CR.

Similar protection against neurotoxic injury has been reported by Duan and Mattson [40] who showed that MPTP-induced loss of dopaminergic neurons and deficits in motor function were ameliorated in CR rats. This protection was also seen in AL-fed rats administered the CR mimetic 2-DG, and was expressed as improved motor performance following MPTP treatment. Since MPTP administration is used to model PD these findings suggest that CR or CR mimetics may be useful tools in the study of this disease as well.

However, not all the data involving the effects of CR in animal models of degenerative disease has been positive. Pedersen et al. [180] examined the effects of APP deposition in mutant APP transgenic mice under CR conditions and found that APP mutant mice exhibited severe hypoglycemia and death following CR. It appeared that the combination of APP mutations and CR-induced reactivity to stress that did not allow them to cope with the mild stress produced by APP deposition.

### 2.5. CR effects on cognitive behavior

Given the benefits of CR in the brain, it is reasonable to assume that these benefits will translate into improved behavioral outcomes for rats following this regimen. Nevertheless, the literature on CR and behavior is rather scarce and at times contradictive, which may illustrate the complexity of the amount of variables that are present during aging.

Various studies have examined cognitive behavior in animals subjected to CR using various tasks, some of which have reported positive effects and others, which have reported negative effects. For example, Beatty et al. [13] examined the effects of CR in animals being fed on alternate days (EOD) on the eight-arm radial arm maze. Subjects were kept on this schedule from 3 to 21 months of age and then were tested on the radial maze. When their performance was compared to that of an aged group fed AL and to 3-month-old controls the authors found that rats fed AL until training adapted to the maze more slowly than young controls and were impaired in acquiring accurate spatial memory. Moreover, it was shown that CR eliminated age-related differences in the initial rate of adaptation to the radial maze but had no effect on the development of accurate spatial memory at any of the retention intervals.

Similarly, Stewart et al. [225] examined the effect of life-long 60% CR on performance in two tasks involving spatial memory, the eight-arm radial maze and the Morris water maze. This study was performed in young and aged food-restricted and AL-fed Fischer 344 rats which were compared at 8, 16, and 24 months of age on both tasks. Results for the eight-arm maze demonstrated that although 24-month-old animals performed more poorly than 8- and 16-month-old animals during the first week of testing, overall accuracy of performance did not vary significantly as a function of age. However, they also found that at the two younger ages, restricted animals performed more quickly than did AL-fed animals. In the Morris water maze, both distance swum and time to find the platform increased with age as well as in aged rats under life-long CR. Nevertheless, CR produced significant improvements in performance in the water maze in aged rats.

Pitsikas et al. [185] used a battery of tests to examine the effects of CR on sensory–motor and cognitive behavior in aged (24 months), adult (12 months), and young (4 months) rats kept on their respective diets from the age of 3 weeks old until testing ended. Results illustrated an age-related deterioration of sensory–motor functions, motility and exploratory activity in all the senescent animals independent of diet. Moreover, AL-fed adults showed some deficits in spatial memory evaluated using the Morris water maze test, as indicated by a slower learning curve. In aged rats, both learning and memory utilization were impaired but these cognitive deficiencies were absent in food-restricted groups.

Striatal function and behaviors associated with this region, such as psychomotor performance and rotational behavior, have been shown to be sensitive to age [92,93,101] and have also been examined under CR paradigms [88,95]. An early study carried out by Joseph and coworkers examined the effects of CR on striatally mediated rotational behavior and stereotypy induced by intrastriatal administration of dopamine-excitatory or cholinergic-inhibitory agents in young and aged rats. The authors found that at low doses of these agents rotational behavior and stereotypy were improved under CR conditions, as shown by declines in these behaviors. However, at high doses animals under CR showed opposite effects, thus illustrating a possible

specificity of effects of dietary restriction on striatal functioning and behavior in senescence.

Similarly, Ingram et al. [88] examined the effects of life-long CR in Female C3B10RF1 mice. The animals were tested on a behavioral battery at 11–15 or 31–35 months of age (middle-aged versus aged) and the authors found that age-related effects observed among control groups in tests of motor coordination (rotorod) and learning (complex maze) were prevented by the CR. Moreover, CR increased locomotor activity in a run-wheel cage among mice of both ages but did not affect exploratory activity in a novel arena.

Nevertheless, some reports suggest that the beneficial behavioral effects of CR, (especially on spatial behavior) are dampened when the regimen is begun later on in life [15]. For example, Means et al. [151] examined the effects of CR in C57BL/6 mice that were placed under this regimen starting at 14 months of age and ending at ages 22 and 25 months, at which point they were given a battery of behavioral tests. The authors found that midlife onset CR increased longevity and preserved strength, coordination, and spontaneous-alternation behavior and altered responses to enclosed alleys. However, spatial discrimination in the Morris water maze and a spatial delayed matching-to-sample water-escape task were insensitive to age and diet.

Similarly, examination of the effects of long-term dietary restriction (60% of ad libitum calories) on age-related alteration of memory and sensorimotor function in Fischer 344 male rats at 6, 12, 18, and 24 months demonstrated that there was a similar decrease in reference memory of calorie-restricted and AL-fed rats at 18 months, and performance deficits in working memory tasks were equally observed in both diet groups at the age of 24 months. This indicates that diet failed to provide protection against age-related deficits in memory [144].

### *2.6. Summary and conclusions*

Close examination of the data reviewed above suggests that CR has dramatic effects on oxidative stress parameters [31,43,57,110,246] and seems to induce neuroprotective mechanisms that are beneficial for neuronal cells and thus may be responsible for the beneficial effects seen in brain [72,126]. CR regimens seem to reduce the production of various free radicals. However, while it is thought that these reductions may be metabolism dependent, based on the studies carried out using 2-deoxyglucose [125,255], an agent that reduces glucose availability and thus mimics certain aspects of CR, some researchers have demonstrated that metabolic rate may not be altered by this regimen [150]; thus, the mechanism by which CR regimens produce their effects are unclear.

Encouraging results of CR are also found in neurodegenerative disease literature; studies using models of AD [180,264] and PD [148] have shown that dietary restriction may ameliorate the neurodegenerative-related symptomatology produced by autosomal mutations or injections of agents that induce these effects. Nevertheless, the studies in this area are few and need replication in order to confirm these findings.

Overall, CR has been shown to successfully modulate a wide array of age-sensitive parameters. However, when examining these studies, two issues become relevant: (1) the magnitude and consistency of these effects, especially in behavioral studies, shows high levels of variability across studies, and (2) that the onset of CR and the duration of the regimen is important in the outcome. Various reports illustrate that the most beneficial effects of CR are induced when the animals are placed under this condition early in life (Table 1). When dietary restriction is begun during mid-life the magnitude of these changes is reduced [28,131,132,151,192,241]. Thus, these results illustrate that while CR has some benefits, its utility as an intervention, especially in humans, may be limited.

## 3. Dietary supplementation

As alluded to above, there has been a great deal of information over the years which suggests that there are increases in vulnerability to inflammatory and oxidative insults in aging (see [75,99,100,133]) and studies reviewed in the previous section indicate that CR may produce its beneficial effects by decreasing the sensitivity to these insults. However, an impressive body of literature also suggests that similar protection may be afforded through alterations in the intake of foods or supplements high in antioxidant activity, and there have been a number of studies examining the putative benefits of antioxidants in altering, reversing or forestalling age-related and degenerative neuronal and behavioral deficits [103,104,121,137,184,190,216,254]. These dietary manipulations have generally been focused upon foods or supplements containing antioxidant compounds, as well as vitamins such as E and C.

### *3.1. Vitamin E*

Vitamin E (Vit. E) has been one of the most studied antioxidants. It is recognized as a major lipid soluble, chain-breaking antioxidant, preventing lipid peroxidation, and protecting membrane integrity. Alpha-tocopherol is the most efficient isoform of Vit. E that provides its antioxidant efficacy [49] and the literature is replete with studies showing the beneficial effects of Vit. E on immune function [23,68,152,199,237], carcinogenesis [65,174,175,177,229], and coronary artery disease [178,239] in aging. However, although there is a long, well-established history between neuronal function and Vit. E in early development (e.g. spinocerebellar degeneration induced via Vit. E deficiency [74]), the results with respect to its efficacy in brain aging have been mixed.

Vit. E deficiency and supplementation appear to be two extremes of the same continuum with respect to altering

vulnerability to oxidative stress. Various studies have demonstrated that Vit. E deficiency leads to increased levels of oxidative stress damage in the brain. For example, in an early study, Lal et al. [117] used chronic Vit. E deficiency to nutritionally induce central nervous system "aging" in young and aged rats. Histological analysis of lipofuscin accumulation demonstrated that Vit. E-deficient animals had higher contents of this lipid peroxidation product in the hippocampus than young and age-matched controls, thus illustrating that the effects of Vit. E deficiency were greater than the effects of aging alone. These findings were found in parallel to behavioral deficits as well as peripheral alterations.

Similarly, in a more recent study, Macevilly and Muller [139] examined parameters of endogenous lipid peroxidation and susceptibility to oxidative stress in neuronal tissues obtained from 1-year-old Vit. E-deficient and control rats. The findings demonstrated that chronic Vit. E deficiency resulted in increased levels of endogenous lipid peroxidation in neural tissues when compared to controls. They also found that the susceptibility of neural tissues to in vitro oxidative stress was increased in Vit. E-deficient rats. It must be said, however, that the levels of susceptibility of different brain regions to in vitro oxidative stress varied, showing the greatest susceptibility in cortex, striatum, and cerebellum.

Conversely, Vit. E supplementation has been associated with the reduction of experimentally-induced or age-associated oxidative stress. In a recent study carried out by Ilavazhagan et al. [87], the authors explored the effect of Vit. E supplementation on various hematological and biochemical parameters in hypoxia-induced oxidative stress in albino rats. Results illustrated that the increases in hematocrit and hemoglobin and decreases in red blood cell deformability were reversed by Vit. E supplementation. Moreover, they also demonstrated that hypoxia-related decreases in plasma Vit. E, blood glutathione (GSH), and increases in plasma malondialdehyde levels were arrested by Vit. E supplementation.

Recently, Kheir-Eldin et al. [107] have evaluated the ability of Vit. E to protect the brain from inflammatory-induced oxidative stress by agents such as lipopolysaccharide (LPS). The administration of LPS injections in young rats resulted in a significant increase in plasma corticosterone, glucose concentration, and changes in brain oxidative status as measured by elevations in lipid peroxidation parameters and reductions of glutathione, as well as disturbances in brain energy metabolism. The authors found that treatment with Vit. E prior to LPS injection ameliorated all the parameters that had been altered by the administration of this endotoxin.

Along these lines, O'donnell and Lynch [169] analyzed changes in enzymatic and non-enzymatic antioxidant levels, in parallel with interleukin-1beta (Il1-β) concentration in cortical tissue prepared from young and aged rats. In this study, the authors found that age-related increases in the activity of SOD, lipid peroxidation and Il1-β concentrations, which are consistent with a compromised antioxidant defense in the cortex of aged rats, were reversed in tissue prepared from rats fed on a diet supplemented with alpha-tocopherol for 12 weeks.

Antioxidants can also function as powerful protectants for neurons in vitro. Behl [14] examined the neuroprotective activity of lipophilic free radical scavengers, synthetic alpha-tocopherol and natural alpha-tocopherol, against oxidative stress and compared their effects to the neuroprotective effect of the female sex hormone estradiol. The author found that both types of alpha-tocopherol had greater neuroprotective effects than 17-β estradiol in mouse clonal hippocampal HT22 cells and rat cerebellar granule neurons. Moreover, he also found that natural alpha-tocopherol protected neurons effectively against oxidative cell death caused by the amyloid-β protein, hydrogen peroxide, and glutamate, and induced the activity of NFK-β, a redox-sensitive transcription factor which is involved in the control of cell survival.

Similar levels of neuroprotection by Vit. E have been shown in age-related oxidative stress parameters. For example, Joseph et al. [98] examined whether oxidative stress sensitivity of aged-animal slices of striatum, a highly oxidizable region due to high levels of dopamine (DA) concentration, could be attenuated by Vit. E supplementation when inducing oxidative stress by incubation with reactive oxidative species. Results indicated that if the striatal tissue was incubated in either Trolox (alpha-tocopherol) or alpha-phenyl-*n*-*tert*-butyl nitrone (PBN), a nitrone spin trap, prior to OS, the negative effects of NO and $H_2O_2$ were reversed in both age groups.

It also appears that the neuroprotection provided by alpha-tocopherol may translate into alterations in neuronal functioning. A recent study showed that impairments in the ability to sustain long-term potentiation (LTP) in the dentate gyrus of aged animals were reversed by Vit. E supplementation. Moreover, histological analysis of the tissue revealed that age-related increases in Il1-β and lipid peroxidation and decreases in membrane arachidonic acid concentration were reversed with this treatment [163].

In addition to its direct effects as a free radical scavenger, Vit. E can also enhance endogenous protective mechanisms by the modulation of transcription factors such as AP-1 (activator protein-1) or nuclear factor kinase-B (NF-KB).

In response to oxidative stress, the expression of a number of genes mediated mitogen-activated protein kinases is increased and followed by stimulation of at least two transcription factors, NF-KB and AP-1. Transcription factors such as AP-1 and NF-KB are responsible for a wide array of extracellular signaling molecules involved in inflammation [122], tissue remodeling, oncogenesis and apoptosis [208], all of which are known to orchestrate many of the degenerative changes associated with aging. Nevertheless, the regulation of these transcription factors is complex. Meanwhile, the activation of AP-1 has been shown to initially act as a potent antioxidant transcription factor [122], and its function, to be further potentiated by antioxidants such as Vit. E [9–11]. It has been hypothesized that the increase of

oxidant overload during aging allows the inflammatory response of AP-1 to overtake its antioxidant response, giving rise to various age-related conditions [122]. Interestingly, during chronic oxidative stress conditions or under models of AP-1 activation, alpha-tocopherol has been reported to regulate AP-1 function as well as NF-κB in the opposite fashion. That is, by decreasing the levels of activation of these transcription factors [53,141] and thus preventing the negative ramifications that are associated with prolonged activation of these transcription factors.

*3.1.1. Vitamin E effects on behavior*

Although sparse, studies concerned with the neuronal changes produced by oxidative stress and the capacity of Vit. E to at least partially reverse the deleterious effects of aging are also evident at a behavioral level. Supplementation with Vit. E has been shown to reverse age-related or oxidative-stress-induced cognitive and motor output in various species.

Ichitani et al. [86] supplemented rats from 1 to 25 months of age with Vit. E and then tested them in a step-through passive avoidance task. In this case, results indicated that the supplemented group exhibited a trend toward higher latencies in this task with respect to control subjects.

More recently, Joseph et al. [104] have examined the effects of chronic Vit. E supplementation on cognitive behavior in aged rats using the Morris water maze task. This study demonstrated that 8-month supplementation with 500 IU/kg of Vit. E resulted in the rats swimming shorter distances to reach the hidden platform, thus replicating previous reports illustrating the benefits of Vit. E supplementation on cognitive behavior.

Evidence of the beneficial effects of Vit. E administration on cognitive behavior is also present in studies carried out in humans. There is evidence to suggest that oral daily supplementation with 300 mg of Vit. E (and 1000 mg Vit. C) for 12 months improves short term memory and motor performance in both males and females [222]. Moreover, people who took Vit. E (30 mg per day) for 6 years or more had higher scores than controls on four of the cognitive measures analyzed, such as visuospatial skills [116].

*3.1.2. Neurodegenerative disease and Vitamin E*

Given that Vit. E can reverse oxidative stress and age-induced deficits in various parameters, that neurodegenerative diseases such as AD and PD are, at least partly, related to oxidative stress and related inflammatory processes [91,181,203,214], and that these diseases are superimposed upon an aged environment, it is reasonable to assume that Vit. E may be capable of reversing or attenuating some of the symptomatology seen in these diseases.

In the case of AD, studies by Sano et al. [200,201] showed that alpha-tocopherol, either with or without selegiline, increased the level of independence in patients and delayed the deterioration of their living performance, but did not improve cognitive test scores. Although subsequent studies have questioned the statistical analyses of these investigations [109,183], there are indications that factors such as the time of treatment initiation might be important in the efficacy of this treatment [232].

Nevertheless, just as seen above with respect to CR, there are indications in pre-clinical investigations that Vit. E can modify the increases in oxidative stress induced via the known pre-disposing factors relevant to AD. For example, Ramassamy et al. [187] have recently examined the oxidant/antioxidant status in hippocampus and frontal cortex of apoE-deficient and wild-type mice at 3 and 13 months and found decreased levels of alpha-tocopherol in apoE-deficient mice at these time points.

Following a similar line of research, Reich et al. [189] examined apoE–antioxidant interactions by quantifying the major isomers of cerebral isoprostanes derived from arachidonic acid (AA) and docosahexanoic acid (DHA) oxidation in aged male and female apoE-deficient and non-deficient mice fed a normal, alpha-tocopherol-deficient, or -supplemented diet. Results indicated that cerebral arachidonic oxidation was decreased by 28% in animals fed the supplemented diet. However, with the alpha-tocopherol-deficient diet, DHA oxidation was increased by 81%. Alpha-tocopherol supplementation decreased enhanced DHA oxidation but did not alter the increases in AA, indicating that dietary alpha-tocopherol effects may be selective with respect to AA and DHA oxidation in the aged male mouse.

In a similar study, Veinbergs et al. [233] determined whether 12-month supplementation with Vit. E in young apoE-deficient mice was neuroprotective and found that the mice that received the supplemented diet showed significantly improved behavioral performance in the Morris water maze. Moreover, improved behavioral output was associated with preservation of the dendritic structure and normal levels of lipid peroxidation and glutathione, when compared to untreated apoE-deficient mice.

In the case of the modification of in vitro A-β toxicity, just as seen with respect to CR [72], Butterfield et al. [22] demonstrated that Vit. E can positively modulate A-β(1–40)-induced oxidative damage to creatine kinase and cellular proteins in cultured embryonic hippocampal neurons.

Similarly, Subramaniam et al. [227] used electron paramagnetic resonance studies of spin-labeled cortical synaptosomal membrane proteins to study conformational changes in proteins, and spectrophotometric methods to examine mitochondrial function, in order to study the effect of Vit. E on samples that were treated with A-β. The results indicated that Vit. E could prevent the toxic effects of A-β in the various parameters measured (e.g. increases in protein carbonyl content).

As mentioned above, calcium homeostasis is also altered by A-β deposition. Huang et al. [83] used cultured cortical cells to study the alterations in calcium homeostasis underlying the neurotoxic effect of A-β and found that Vit. E was effective in preventing the A-β-induced increases in cytosolic free calcium ($Ca_i^{2+}$). In addition, Vit. E was also

effective in preventing the subsequent cell death induced by this peptide, as assessed via several methods including lactate dehydrogenase release.

The effects of A-β neurotoxicity and the capacity of Vit. E to block these effects has also been demonstrated in vivo. Yamada et al. [248] have demonstrated this neuroprotective capacity by examining a battery of behaviors during continuous intracerebroventricular infusion of A-β(1–42). In this study, the authors found that Y-maze and Morris water maze performance were significantly impaired in A-β-infused rats when compared to control rats. However, the repeated daily administration of alpha-tocopherol orally 3 days before the start of A-β infusion to the end of testing prevented the behavioral deficits in both tasks, suggesting that treatment with antioxidants such as idebenone and alpha-tocopherol prevent learning and memory deficits caused by A-β. Thus, although there are few pre-clinical mechanistic studies, it would appear that there might be some benefit of Vit. E supplementation against A-β toxicity or the enhanced sensitivity to oxidative stress seen in apoE-deficient animals. Whether these findings can ultimately translate into clinical efficacy in AD awaits further specification. Note, however, that methodological problems notwithstanding, there is some evidence from the studies of Sano et al. [200,201] studies of the clinical efficacy of Vit. E in AD, at least initially.

Just as there is an oxidative stress component in AD, it also appears, as pointed out in the previous section, that oxidative stress is a major contributor to selective neuronal death in PD [108,203,260]. Thus, it is reasonable to hypothesize that Vit. E supplementation would also ameliorate the sympotomatology of PD. Indeed, Roghani and Behzadi [195] have recently investigated the neuroprotective effect of Vit. E in a standard model of PD, which employs unilateral intrastriatal 6-hydroxydopamine (6-OHDA) lesions and assesses post-lesion apomorphine- and amphetamine-induced rotational behavior. The results indicated that there were 74 and 68% reductions in contraversive and ipsiversive rotations in the E-injected group, respectively, as compared with the vehicle-injected group. Tyrosine hydroxylase-immunohistochemical analyses showed that the Vit. E-pretreated group had a reduction of 18% in ipsilateral substantia nigra pars compacta (SNC) cells, while the cell number dropped 53% in the non-treated lesioned group. In addition, retrograde-labeled neurons in ipsilateral SNC were reduced by up to 30% in the pretreated group while the non-pretreated group showed reductions of 65%.

To investigate the possible role of Vit. E in PD in humans, both epidemiological and intervention studies have been undertaken. The former investigated the correlation between the intake of antioxidants and the incidence of PD, while the latter examined the putative benefits of Vit. E in PD. The results of both types of studies have been mixed. Surveys of early-life intake of various foods showed that subjects that ate foods particularly rich in Vit. E were less likely to develop PD in later life [63,64]. Similar findings were reported in an additional study that assessed PD incidence and the intake of supplemental Vit. E [35]. However, other studies have failed to find any type of association between amount of Vit. E intake and incidence of PD [78,134,204].

Clinical examinations have shown that the administration of high doses of both Vit. C (3000 mg per day) and Vit. E (3200 IU per day), when administered to PD patients 1–8 years prior to the onset of levo-dopa (L-dopa) treatment, delayed the need for this pharmacological treatment by 2–3 years [50,52]. Again, however, in additional clinical assessments in which Vit. E (2000 IU per day) was combined with selegiline (a monoamine oxidase inhibitor), effects were observed only with selegiline but not Vit. E, and no effects of the treatment were seen in motor or cognitive performance [1–5]. It should be noted that numerous methodological differences among the various studies should be examined further to determine the necessary conditions for the beneficial effects of Vit. E to be observed.

### *3.1.3. Summary and conclusions*

From the literature reviewed above, it appears that Vit. E has some protective effects in reducing the damage created by oxidative stress and appears to be effective in aging and in reducing toxicity to putative ROS-generating agents such as A-β. Moreover, studies reviewed above show that the benefits of Vit. E supplementation may be age-dependent and are observed in senescence, perhaps in some instances of age-related neurodegenerative diseases. However, it is clear that the onset, dose and duration of the Vit. E supplementation is of paramount importance.

Also relevant in this discussion is the fact that while Vit. E may not exert full benefits on patients with degenerative disease a combination of antioxidants may be more effective in forestalling these symptomatologies. A recent study by Kontush et al. [113] partially validates the findings of Sano et al. [200,201]. Kontush and coworkers recently supplemented 10 AD patients/group daily for 1 month with either a combination of 400 IU Vit. E and 1000 mg Vit. C, or 400 IU Vit. E alone. The results showed that the susceptibility of CSF and plasma lipoproteins to in vitro oxidation was significantly decreased in the group given the combination of Vit. E and C. In contrast, although the supplementation with Vit. E alone resulted in higher concentrations of this agent in CSF and plasma, no decreases were observed in lipoprotein oxidizability. Clearly, the combination of antioxidants (Vit. C and E) was more effective in providing antioxidant protection. Similarly, in the studies by Fahn [50,51], the Vit. E and C combinations delayed the onset of the L-dopa treatment. In this respect, combinations of antioxidants might provide greater protection than that afforded by a single antioxidant, and this might be especially true in the case of the plethora of phytochemical antioxidants in fruits, vegetables and herbals. Nevertheless, at least in the models and experimental conditions discussed above, Vit. E appears to be as effective as CR in providing in vivo and in vitro antioxidant protection.

As for related single antioxidants such as Vit. C and β-carotene, studies have been mixed and it has been difficult to ascertain from many of the studies their role in impaired cognition and brain aging. Many of these studies and additional ones are listed along with their findings in Youdim and Joseph [254].

### 3.2. Phytochemicals

From the above discussion, it could be postulated that greater benefits might be achieved with respect to brain aging and neurodegenerative disease through the administration of combinations of antioxidants/anti-inflammatories. One method that may accomplish this is the increased dietary intake of foods or herbals that are high in antioxidants. Plants, including food plants (fruits and vegetables), synthesize a vast array of chemical compounds that are not involved in their primary metabolism. These 'secondary compounds' instead serve a variety of ecological functions ultimately to enhance the plant's survivability and may be responsible for the multitude of beneficial effects of fruits and vegetables on an array of health problems, two of the most important of which may be their antioxidant and anti-inflammatory properties.

Since oxidative stress and inflammation appear to be involved in the signaling and behavioral losses seen in senescence, an important question becomes whether increasing antioxidant intake would forestall or prevent these changes, and there are numerous studies in which a large variety of polyphenolic dietary agents have been employed to alter behavioral and neuronal deficits with aging, such as garlic (reviewed in [254]), herbals (e.g. ginseng or Ding Lang, reviewed in [24,254]; tea [254]; and *Ginkgo biloba*, which has been shown to have beneficial effects on behavioral aging and perhaps AD, see [30,36] for reviews). In fact, the standardized extract of *Ginkgo biloba*, EGb761, has been the subject of intense study. It contains numerous flavonglycosides and proanthocyanidins among other compounds. EGb761 is composed of 24% flavonoid glycosides; rutin, the glycoside of quercetin, accounts for 11.1%. In addition to flavonoids, EGb761 consists of unique terpenes (3% bilobalide and 3% ginkgolides A, B, and C) [39]. Thus, we will briefly review *Ginkgo biloba*'s beneficial properties here to illustrate the effectiveness of the polyphenolics in brain aging and behavior.

#### 3.2.1. Gingko biloba

EGb761 has been used in the treatment of multiple diseases including cardiovascular and cerebrovascular disease, all of which have components associated with oxidative stress [235]. In vitro, it is a potent free radical scavenger and inhibitor of NADPH-oxidase, which significantly decreases superoxide radical, hydrogen peroxide, and hydroxyl radical production in human neutrophils stimulated with phorbol ester [142]. Indeed, several studies have shown this to be the case. For example, Oyama et al. [176] examined the effect of *Ginkgo biloba* extract on dissociated rat cerebellar neurons suffering from oxidative stress induced by hydrogen peroxide and found that this treatment increased the number of ethidium neurons stained in a time-dependent manner. However, following pretreatment with Gingko biloba extract the time-dependent increase in the number of dead neurons during exposure to $H_2O_2$ was significantly delayed.

These findings were replicated in a later study by Xin et al. [247], in which the protective effects of this extract was studied on apoptosis in cerebellar granule cells produced by treatment with $H_2O_2/FeSO_4$. This treatment in the absence of EGb761 produced decreases in the Bcl-2 mRNA levels and an increase in C Fos and C Jun. However, these decrements were attenuated by EGb761, or a mixture of flavonoids and terpenes, but not by terpenes alone.

Similar findings were also reported by Bastianetto et al. [12] in hippocampal cell cultures. Their findings showed that the effects of oxidative stress were antagonized by either EGb761 or its flavonoid fraction, CP 205, but not by the terpenoid constituents of EGb761. Moreover, EGb761, CP 205 and chelerythrine were also able to rescue hippocampal cells pre-exposed to sodium nitroprusside. Pre-treatment with EGb761 was also shown to be protective in neuronal cultures exposed to MPTP [176], but not 6-hydroxydopamine.

More recently Bridi et al. [20] have examined the capacity of EGb761 to modulate endogenous lipid peroxidation and the activity of the endogenous antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD), in the hippocampus, striatum and substantia nigra (SN) of rats. In this study, the authors found that EGb761 increased the CAT and SOD activities in the hippocampus, striatum and SN, and decreased lipid peroxidation in the hippocampus.

Extracts of *Ginkgo* leaves have also been found to be protective of free radical insults induced by experimental manipulations in vivo. For example, Oberpichler et al. [168] examined the effects of *Ginkgo* extract on free-radical damage produced by hypoxia. In this study, the authors compared the effects of the flavone and the non-flavone fraction of EGb761 with those of the whole extract in mice undergoing lethal hypoxia (3.5% $O_2$), on brain energy metabolism, and on local cerebral blood flow (LCBF) of normoxic rats. They found that EGb761, as well as its non-flavone fraction, considerably prolonged the survival time of mice under lethal hypoxia. Moreover, the authors also found that EGb761 retarded the breakdown of brain energy metabolism in the hypoxic, artificially-ventilated rats. Lastly, both non-flavone and EGb761 treatment increased LCBF in the majority of 35 examined brain regions while the flavone fraction caused only minor alterations of LCBF.

#### 3.2.2. Ginkgo biloba effects on behavior

In a similar fashion to supplementation with Vit. E, supplementation with *Ginkgo biloba* extract has been shown to produce beneficial effects on behavior. An early study performed by Winter [243] demonstrated that acquisition, performance, and retention in mice during an operant

conditioning task was facilitated when the subjects were supplemented with EGb761 daily, at a dose level of 100 mg/kg orally for 4–8 weeks. More recently, Winter [244] examined the effects of this supplementation on eight-arm radial maze performance. Chronic administration of EGb761 at a dose of 50 mg/kg in sweetened condensed milk five times per week from 7 months of age had no effect on continuous learning, but the same dose resulted in a trend toward fewer sessions to reach criterion. In addition, findings indicate that the supplemented animals made fewer errors than their non-supplemented counterparts. When examining delayed non-matching to position performance in aged rats, the administration of EGb761 resulted in a dose-dependent decrease in total, retroactive, and proactive errors. Interestingly, a "CR-like" effect was observed in the rats supplemented with the EGb761 in that they lived significantly longer than the control rats.

The beneficial effects of *Ginkgo biloba* on behavior have also been demonstrated when using passive avoidance paradigms. When EGb761 was given to young, middle aged and aged rats for a 3-week period, the authors demonstrated that aged subjects performed indistinguishably from younger subjects in passive avoidance learning tasks; however, these effects occurred in the absence of membrane fluidity amelioration in the older group [226].

### 3.2.3. Neurodegenerative disease and Ginkgo biloba

Based on the encouraging literature illustrating the capacity of *Ginkgo biloba* extract to attenuate oxidative stress parameters and ameliorate behavioral output in aged rats or in humans, there is increasing interest in examining the effects of this extract on neurodegenerative disease such as AD. A recent study carried out by Ramassamy et al. [187] examined the protective ability of EGb761 and other antioxidants in tissue of AD patients against oxidative stress insults. The authors found that EGb761, the neurosteroid dehydroepiandrosterone (DHEA) and human recombinant apoE3 (hapoE3rec) were able to protect control, AD APOE-3/3 and APOE-3/4 cases against hydrogen peroxide/iron-induced lipid peroxidation. Nevertheless, EGb761 and DHEA had no effect in homozygous E4 cases.

*Ginkgo biloba* has also been shown to be effective in decreasing neurotoxicity associated with A-β toxicity. Zhou et al. [262] examined the effect of bilobalide, a terpene extracted from the leaves of *Ginkgo biloba*, on the A-β peptide fragment 25–35 (A-β 25–35)-induced PC12 cell cytotoxicity, and found that decreases in cell viability after 24 h treatment with A-β were dose-dependently attenuated by bilobalide. Moreover, bilobalide also inhibited A-β 25–35 (100 μmol/l)-induced elevation of lipid peroxidation and decline of antioxidant enzyme activities. EGb761 also rescued the PC12 neuronal cells from A-β-induced cell death by inhibiting the formation of A-β-derived diffusable neurotoxic ligands.

Similarly, Yao et al. [251] have recently demonstrated that pre-treatment of PC12 cells with EGb761 prevented, in a dose-dependent manner, the increases in free radical production, glucose uptake, apoptosis and cell death induced via A-β exposure. Based on these results the authors also examined whether EGb761 interacts directly with A-β and found that EGb761 inhibits, in a dose-dependent manner, the formation of A-β-derived diffusable neurotoxic soluble ligands (ADDLs), suggested to be involved in the pathogenesis of AD.

Similar results have been shown in primary cultured cells. For example, Bastianetto et al. [12] have shown that EGb761 concentration-dependently protected hippocampal neurons against toxicity induced by A-β fragments. Moreover, EGb761 was able to protect (up to 8 h) hippocampal cells from pre-exposure to A-β 25–35 and A-β 1–40.

### 3.2.4. Ginkgo biloba effects in humans

Because of the association of AD with injuries due to reactive oxygen species injuries and the beneficial effects of EGb761 on cognitive behavior in animals, the action of EGb761 as an antioxidant has been tried as a potential treatment for Alzheimer's type dementia.

Le Bars et al. [124] assessed the efficacy and safety of EGb761 in AD and multi-infarct dementia by carrying out a 52-week, randomized double blind, placebo-controlled, parallel-group, multi-center study. The patients in this study, who were mildly to severely demented outpatients with Alzheimer disease or multi-infarct dementia, were randomly assigned to treatment with EGb761 (120 mg per day) or placebo and evaluated using various cognitive and social functioning scales shown to be sensitive in AD. In this study, EGb761 was concluded to be safe and capable of stabilizing and, in a substantial number of cases, to improve the cognitive performance and the social functioning of demented patients for 6 months to 1 year after treatment.

In a follow-up study, the same group [123] carried out an intent-to-treat (ITT) analysis to provide a realistic image of the efficacy that could be expected after 26 weeks of treatment with EGb761. The data collected during the previous study showed that 76% of the patients on placebo and 73% of those supplemented with EGb761 reached the 26th-week visit. In comparison to the baseline values, the placebo group showed a statistically significant worsening in all domains of assessment, while the group receiving EGb761 was considered slightly improved on the cognitive assessment and the daily living and social behavior.

Other studies have examined the effects of this extract on patients with various degrees of AD symptomatology during performance of various psychometric tasks. In one study [145], the effects of EGb761 on AD type dementia were examined using a placebo group and EGb761-treated group (240 mg/3 times a day/3 months). The experimenters used the SKT-test (a battery of cognitive performance tasks) to assess the patient's attention and memory, a multiple vocabulary test to examine pre-morbid general intelligence levels of the patients, a trail making test, and assessment with the ADAS questionnaire. Analysis of the data showed that

3-month treatment with this extract showed significant improvement in the SKT test and trends toward improvement in the trail making test and ADAS score.

#### 3.2.5. Summary and conclusions

Thus, the data with respect to EGb761 and its polyphenolic compounds, just as was seen with respect to Vit. E, have shown significant positive protective effects against both in vivo and in vitro oxidative stress, and they further indicate that there is some efficacy of EGb761 in cognitive dysfunction in aging and AD. Importantly, it also appears that the polyphenolics may have some similar benefits to those seen with respect to CR in that supplementation with EGb761 may increase longevity. In relation to longevity and supplementation with combinations of antioxidants, Bezlepkin et al. [16] have demonstrated that supplementation with a combination of β-carotene, alpha-tochopherol, ascorbic acid, rutin, selenium and zinc, given to C57/Bl/6 mice starting early in life (2–9 months) significantly increases survival (86–105 days) and mortality age by 9.5%. Clearly, a great deal of more work is needed in this area but the findings reviewed above indicate that by taking advantage of the putative benefits of the dietary supplementation of the plant-based polyphenolic compounds, some of the deleterious effects of brain aging may be reduced or forestalled. If this is the case, one question that could be asked may be whether the phytonutrients in fruits and vegetables would have similar effects.

### 3.3. Dietary supplementation of antioxidants through foods

There are over 4000 polyphenolic structures that occur in fruits, vegetables, nuts, seeds, and grains [140]. As briefly mentioned above, they have been shown to possess antioxidant, anti-allergic, anti-inflammatory, anti-viral, anti-proliferative, and anti-carcinogenic activities [47,48,58,80,154,155]. The literature is replete with studies showing that the combinations of polyphenolic compounds found in fruits and vegetables may reduce the incidence of cardiovascular and cerebrovascular diseases [7,85,149,234] and cancer [38,242]. Even lycopene extracts from single foods such as tomatoes have been reported to have anti-tumor properties [207].

It has been known for several years that supplementation with only one antioxidant may not be optimal [129], as more than one of several endogenous antioxidants may be affected during disease [8,70,217]. Moreover, some antioxidants may be more effective than others at scavenging a variety of ROS than others, again suggesting that it is the combination of the significant number of polyphenolics present in the fruits and vegetables that may be responsible for the ubiquitous beneficial effects observed.

However, until recently, few studies had examined the effects of fruits and vegetables on CNS aging and degenerative disease. One such vegetable is garlic, which is known for its beneficial properties at a peripheral (e.g. cancer, see [118] for review), as well as at a CNS level. For example, aged garlic extract (*Allium sativum*, which contains *S*-allycisteine, *S*-allymercaptocysteine, allicin and diallosulfides) administered at 2% (w/w) of the diet was shown to exhibit beneficial effects towards cognitive impairments in a novel strain of senescence accelerated mouse (SAM) [159,160,166,261]. Although this treatment did not affect motor activity, there were additional beneficial effects of the treatment reflected in an increased survival rate, less atrophy in the frontal brain areas, and enhanced performance in both passive and active avoidance, which the authors attribute to the antioxidant properties of the aged garlic extract.

Although there have not been evaluations of antioxidant activity in vivo, but only tested in vitro, garlic extract has also been shown to protect against oxidative stress-induced increases in thiobarbituric-acid-reactive substances (TBARS) [81,82], and to promote the survival of neurons derived from various regions of the neonatal brain (increased neurogenesis) [161]. Until these effects are tested in the whole animal, and until the aged garlic extract components are actually isolated and identified within the brain, the mechanisms involved in its protective effects are difficult to determine.

Additionally, it is also important to determine the total antioxidant capacity of the foods under study. One method is through the use of the oxygen radical absorbance capacity (ORAC) procedure [25,26,238]. Various fruits and vegetables ranging from strawberries to spinach have been shown to have a high antioxidant capacity using this procedure. Based on this evidence it is reasonable to hypothesize that supplementation with fruits and vegetables will affect oxidative stress parameters in a similar fashion to that of Vit. E or *Ginkgo biloba*. Moreover, and in a similar fashion to that of Gingko biloba, the protection seen by fruits and vegetables may be produced by the antioxidant capacity of flavonoids found in these foods.

Even though the literature in this field is not as extensive as that on the compounds described above, research indeed suggests that fruits and vegetable high in antioxidants do show similar properties to those of Vit. E and *Ginkgo biloba* and that these effects may be mediated by antioxidant flavonoid action. For example, Kawai et al. [105] examined the effects of Manda, a product prepared by yeast fermentation of several fruits and black sugar, high in various flavonoids, on lipid peroxidation in the senescent rat brain by inducing hydroxyl radical activity via a $FeSO_4$–$H_2O_2$ system. The authors found that addition of Manda to brain homogenates of adult rats, incubation of brain homogenates with Manda for 2 and 3 h, and oral administration of Manda all suppressed the age-related increase in lipid peroxidation in the hippocampus and striatum but not in cerebral cortex.

Another vegetable found to have antioxidant and anti-inflammation properties is the broad bean, a fava bean rich in various free radical scavengers. Okada and Okada [171] isolated the radical scavenger “water soluble protein” (WSP) from broad beans and examined their capacity to increase

various endogenous antioxidants. In this study, cells were treated with WSP or hydrocortisone (also shown to inhibit lipid peroxidation) for 4 and 6 weeks in young cells, and for 3 and 6 weeks in old cells, and found that both treatments increased the endogenous activity of catalase as well as the GSH concentration.

While it may be too early to identify the particular phytonutrients in fruits and vegetables that are responsible for these beneficial effects, there have been a few studies which have examined the characteristics of some of the most effective polyphenolics. For example, Ishige et al. [89] have recently examined potential protective mechanisms of flavonoids in cell death in mouse hippocampal cell line HT-22, using a model system in which exogenous glutamate inhibits cystine uptake and depletes intracellular glutathione (GSH), leading to the accumulation of ROS and an increase in $Ca^{2+}$ influx, which ultimately causes neuronal death. Findings indicated that many, but not all, flavonoids protect HT-22 cells and rat primary neurons from glutamate toxicity as well as from five other oxidative injuries. They also found that at least three structural requirements of flavonoids must be present for protection from oxidative stress injury: (a) hydroxylated C3, (b) an unsaturated C ring, and (c) and hydrophobicity. An earlier study [130] had shown that the requirements for effective antioxidant activity and the ability of the flavonoids to protect PC-12 cells could be defined by the number of hydroxyl groups on the phenolic rings.

Similarly, Youdim et al. [252] showed that the anthocyanin class of flavonoids derived from blueberries had greater antioxidant effects and cell-penetrating ability (in erythrocytes) than hydroxycinnamic acids. Along these same lines, Ishige et al. [89] showed that flavonoids used various mechanisms of protection such as increasing intracellular GSH, directly lowering levels of ROS, and preventing the influx of $Ca^{2+}$ despite high levels of ROS. These findings suggest that the mechanism of protection from oxidative insults by flavonoids may be highly specific for each compound. That may explain why, as discussed below, a combination of various flavonoids from fruits and vegetables may be especially powerful in altering the deleterious effects of brain aging and nutritional deficiencies.

As an example of the latter, a recent study [250] has examined the capacity of polyphenols to reverse some of the damage produced by Vit. E deficiency. In this study, the authors evaluated the effect of crude polyphenols (CLP) from cacao liquor on Vit. E-deficient rats. Supplementation of the Vit. E-deficient diet with CLP for 7 weeks was found not to prevent the decrease in alpha-tocopherol levels in the liver, kidney, heart, and brain. Moreover, plasma and tissue lipid peroxide increased in the group fed the Vit. E-deficient diet when compared to the control group. However, these changes were shown to act dose-dependently as a result of supplementation of the Vit. E-deficient diet with CLP. That is, increased levels of lipid peroxide in plasma in the Vit. E-deficient diet group tended to be suppressed as a result of supplementation of the diet with CLP; nevertheless, the differences did not reach statistical significance.

The effects of fruit and vegetable antioxidant activity have also been shown in aging. In this case, the authors chose to examine the changes in response sensitivity of muscarinic receptors (mAChR) that have been shown to occur as a function of age and are sensitive to oxidative stress [91,98]. These changes are expressed downstream as reductions in inositol-tris-phosphate ($IP_3$) levels (see [138,197] for review), and upstream as decrements in losses in carbachol-stimulated GTPase activity (G protein coupling/uncoupling [249]) and oxotremorine-enhanced striatal dopamine release (OX-$K^+$-ERDA) in the aged rodent. The loss of mAChR sensitivity is exacerbated in AD and under oxidative stress (see [59,197] for reviews). It also appears that there are similar decrements in GTPase signaling in DA receptors [97]. Using these parameters, Joseph et al. [104] examined whether long-term feeding of Fischer 344 rats, beginning when the rats were 6 months of age and continuing for 8 months, with diets supplemented with a strawberry or spinach extract (which had been identified by the ORAC assay as being high in antioxidant activity), could prevent the age-related loss of mAChR sensitivity by assessing carbachol-stimulated GTPase activity and OX-$K^+$-ERDA. In addition, they also assessed cognitive performance on the Morris water maze. The results indicated that when given control diets or those supplemented with strawberries or spinach extracts, or Vit. E, the spinach-fed rats demonstrated the greatest retardation of age-effects on all parameters except GTPase activity, in which strawberry extract had the greatest effect. Thus, phytochemicals present in antioxidant-rich foods such as spinach may be beneficial in retarding functional age-related CNS and cognitive behavioral deficits.

Cerebellar beta-adrenergic receptor sensitivity, hippocampal $Ca^{2+}$ buffering capacity, motor and Morris water maze performance also show deficits in aging [17,119,188]. In this regard, it has been demonstrated that supplementation with various antioxidant-rich fruits and vegetables reverses age-induced declines in beta-adrenergic receptor function in cerebellar Purkinje neurons. The authors found that the spinach diet improved learning on a runway motor test, a task modulated by cerebellar norepinephrine, and the MWM, a task modulated by aging, thus suggesting age-related deficits in motor learning and memory can be reversed with nutritional intervention [18].

Similarly, Joseph et al. [103] found that dietary supplementation (for 8 weeks) with spinach, strawberry or blueberry (BB) extracts in an AIN-93 diet was effective in reversing age-related deficits in neuronal and behavioral (cognitive) function in aged (19 months) F344 rats. However, only the BB-supplemented group exhibited improved performance on tests of motor function. Specifically, the BB-supplemented group displayed improved performance on two motor tests that rely on balance and coordination (rod walking and the accelerating rotarod), while none of the

other supplemented groups differed from control on these tasks. All diet groups, but not the control group, showed improved working memory (short-term memory) performance in the Morris water maze, demonstrated as one-trial learning following the 10 min retention interval. Significant increases in several indices of neuronal signaling (e.g. MAChR sensitivity) and reversals in age-related "dysregulation" in $^{45}Ca$ buffering capacity were also found. Examinations of ROS production in the brain tissue obtained from animals in the various diet groups indicated that the striata obtained from all of the supplemented groups exhibited significantly lower levels of ROS activity (via DCF, 2′,7′-dichlorofluorescin diacetate, assessments) than the controls.

A subsequent study using a BB-supplemented NIH-31 diet replicated the previous findings [253]. However, it was clear from these supplementation studies [103,253] that the significant effects of the supplements, especially the BBs on both motor and cognitive behavior, were due to a multiplicity of actions, in addition to those involving antioxidant and anti-inflammatory activity.

Given the efficacy of the BB supplementation as compared to the spinach and strawberry supplements, examinations were carried out in an attempt to determine any additional beneficial effects of BB supplementation on various neuronal parameters. Preliminary findings indicate that supplementation with this fruit may: (a) modulate neurogenesis in the hippocampus [61], (b) increase the protection against the in vitro application of the inflammatory agent, TNF-α, on the expression of HSP-70, and (c) restore the HSP-70 response to inflammatory stress in senescent animals [60].

Moreover, BB supplementation from weaning to 12 months of age in mice transgenic for amyloid precursor protein (APP) and PS1 mutations showed region-specific up-regulation of PKC-α and ERK 1/2 activities in brain regions linked to cognitive behavior (Y-maze performance) [37], and increased levels of expression of calcium-dependent PKC-δ, an enzyme that has been linked to spatial memory [153]. Similarly, it was also demonstrated that BB supplementation increased carbachol-stimulated GTPase in the hippocampus and striatum [37]. These parameters showed significant correlations with Y-maze performance in that increased expression of these signaling agents was associated with enhancements in this behavior. Therefore, it could be speculated that there might be direct effects on signaling by the polyphenolics in the BB that may occur independently of the antioxidant or anti-inflammatory effects. This possibility is being further investigated, but it is clear that there may be a plethora of positive effects of these phytonutrients in both aging and, thus far, at least one model of AD.

### 3.4. Conclusions

Compared to the variety of nutrients present in the diet, phytochemicals have been the subject of relatively little research on the effects of these nutrients on age-related and oxidative stress-induced neuronal changes and behavior. Most studies have been performed on herbal extracts such as *Ginkgo biloba* or even garlic extract (see citations in the previous sections for reviews). However, the fact that these extracts not only affect oxidative stress and inflammatory parameters but also affect more general central nervous system activity such as neurotransmitter function [124], blood viscosity and blood flow properties [182], signaling [37] and anti-stress action [158] suggests that the deleterious effects of aging may be slowed down through the intake of appropriate nutrients.

One event that becomes evident early on is the fact that various components of these fruits and vegetables attenuate different types of oxidative damage. As seen in the studies examining the effects of *Ginkgo biloba*, for example, the flavonoid fractions were better at attenuating lipid peroxidation [12] while the terpenoid constituents were more effective in reversing damage produced by A-β [262]. Moreover, as shown by Ishige et al. [89], flavonoids, a "family" of active ingredients in fruits and vegetables, can modulate oxidative stress damage via a wide array of mechanisms.

Based on this evidence, it seems plausible to assume that the complex interaction of the substances in foods such as fruits and vegetables may be much more effective and much less expensive than the innumerable variety of anti-aging supplements found in today's market that are reported to contribute to "successful aging". The question still arises, though, as to whether it might be more expedient to achieve "successful aging" through a diet containing an abundance of fruits and vegetables, especially those that are high in antioxidant activity, than via CR.

## 4. General discussion: CR versus phytonutrients

From the literature reviewed above, it is clear that oxidative stress plays a definite role in aging and neurodegenerative disease. Both CR and phytonutrients can reduce oxidative stress damage that is experimentally induced or age-related. However, what is also evident is that both interventions: (a) are effective in reducing age-related free-radical induced damage, and (b) can alter, forestall, or reverse many of the deleterious effects of aging. Thus, the goal then becomes not to find which intervention is better but which one is more easily applicable.

While there is a wide diversity of findings described in antioxidant supplementation studies, possibly due to the many doses of antioxidants used, the varying modalities of administration, time course of studies, and variety of methodologies, most studies demonstrated that dietary antioxidants play a role in preventing and slowing down age-related neuronal and behavioral impairments related to insults by reactive oxygen species. Moreover, while the mechanisms by which fruits and vegetables work have not been established, it is clear from epidemiological data that diets rich in antioxidants play a pivotal role in maintaining

health [55]. In addition, the flavonoids in fruits and vegetables can modulate oxidative stress damage via a wide array of mechanisms and are involved in other physiological actions, such as reduction of inflammatory responses [115,157]. Furthermore, the risk of side effects or adverse reactions, by eating a diet that is high in fruits and vegetables, is virtually null. This is an extremely important consideration, since it suggests that many of the adverse effects of aging can possibly be mitigated dietarily with very little risk. Furthermore, while supplementation with one antioxidant (Vit. E) has not been shown to increase longevity [162], research suggests that Vit. E given in combination with other antioxidant agents may increase survival in rodents [16]. More importantly is the fact that, as pointed out above, it may be possible to increase longevity with polyphenolics (EGb761) [245].

As we have reviewed in the case of phytonutrients, there are also a great deal of data outlined in numerous reviews (e.g. [146]) showing that CR has the capacity to attenuate age-related damage, and therefore, as a procedure to study the mechanisms of aging (e.g. the role of free radicals, energy metabolism) it is a powerful tool. However, if the issue becomes one of extending this intervention to humans as a means to reverse or forestall the deleterious effects of aging, there are several important considerations which are as follows.

### 4.1. Compliance

As is well known, the popular media contains numerous references to diets of various kinds, and at the last examination there were almost 2500 citations in Medline for diet and weight loss. Despite this, the proportion of people in the US who are obese continues to rise. For example, Nicklas et al. [165] have reported that one in four children in the US is overweight and 11% are obese. These children have a 1.5–2-fold higher probability for becoming overweight as adults. Additionally the increases in weight and obesity that were assessed from 1983 to 1994 increased by 50% over that found from 1973 to 1982. The question that arises from these considerations is that, given all of the diets and other aids available, why is obesity such a problem? The answer is compliance. If this is the case how would one go about getting people to comply with a lifestyle change where their caloric intake would drop by 20–30%? More importantly what foods would they include in such a calorically restricted diet? Anderson and coworkers have reviewed studies showing that higher fat diets (where weight loss is possible) increase serum cholesterol and, thus, coronary heart disease (CHD), while diets low in saturated fat would lower serum cholesterol and risks for CHD. It must be remembered in nearly all of the CR studies that only the calories were restricted and the diet composition was well controlled for the amount of vitamins, minerals, etc. So, in a human what would comprise the restricted diet that is allowed? Would the saturated fat, fiber, etc. also have to be controlled?

### 4.2. Effectiveness

Similarly, it is difficult to establish the effectiveness of CR in humans. CR has been shown to ameliorate cognitive performance in laboratory animals [13,185]. Nevertheless, when one takes into account that laboratory animals housed under standard conditions tend to eat more than animals housed in other environments [135] and the fact that high caloric intake has been shown to also impair some cognitive function [135], it may be possible that the behavioral amelioration seen in diet-restricted animals is not due to this intervention but due to a reduction in excessive food intake. In this regard, it has been shown that reduction of food intake and dieting in humans leads to decreased cognitive performance [66,77]; thus, chronic dietary restriction may lead to negative mood and lower cognitive performance, hence clearly not enhancing quality of life.

More importantly, dietary restriction in humans has been associated with bingeing [170,186,240]. Corwin et al. [33] have demonstrated that if a preferred food is withheld for a period of time and then presented for a restricted amount of time, rats will increase the amount of intake during these times and will compensate for the excess energy consumed for up to 48 h. Thus, limiting access to preferred foods may contribute to the development of aberrant binge-type behaviors and may end up jeopardizing the beneficial effects of CR.

Additionally, Das et al. [34] have found that resting energy expenditure was significantly attenuated in aged animals after a 6-week hypocaloric diet. These animals showed greater subsequent weight loss when compared to younger subjects and exhibited an impaired ability to regulate food intake for at least 6 months following the hypocaloric diet. If one considers that total energy intake may vary (decrease) by 1000–1200 kcal in men and 600–800 kcal in women, then it may be that for some nutrients older Americans are only receiving 1/5 to 1/3 of the recommended dietary allowance [236]. Thus, what would be the result of recommending CR in a middle-aged or aged group? Note that for the Joseph et al. [103] and Joseph and Youdim [254] studies, the supplementations were begun in senescent animals and significant effects were observed. Would the same be true for CR in either humans or animals? As shown in Table 1 of this review, the overwhelming majority of studies examining CR effects in aging parameters place the animals on this regimen soon after weaning. Moreover, Lipman et al. [131,132] has demonstrated that the beneficial effects of CR are lost if the regimen is begun during mid-life or early senescence. Thus, it seems that supplementation with fruits and vegetables may be more effective than CR when the intervention is begun later on in life.

Various epidemiological reports have been cited as evidence for the beneficial effects of dietary restriction in humans [21,79]. Nevertheless, these data can easily be interpreted in different ways. For example, Bronner cites lower calorie intake in China and Japan as an explanation

for lower incidence of AD; nevertheless, it is well known that Asian diets are particularly high in phytoestrogens (soy products) [164] and omega-3 fatty acids (fish) [172], both of which have been shown to be potent antioxidants [69,231] and beneficial in aging [193]. Thus, it is difficult to assess whether decreased incidence of AD in these countries is due to CR or higher intake of antioxidants.

Similarly, with regard to lower incidence of AD in non-industrialized countries, it is known that less-industrialized countries rely to a higher degree on agricultural products (fruits and vegetables) and less high-fat, processed foods than do inhabitants of industrialized countries [27,127]. Thus, once again, it is difficult to examine these data as convincing evidence for the beneficial effects of CR in humans.

### 4.3. The hormesis effect

As pointed out earlier in this review CR has also been hypothesized to act via a priming of the stress response in these animals. That is, mild stress resulting from CR primes stress responses, in particular neuroprotective responses induced by stress, which then protect the central nervous system from additional insults. While the data described above does validate this hypothesis, paradoxically, CR has also been shown to increase the levels of glucocorticoids, which are known to cause a wide array of disturbances both peripherally and centrally [71], but especially in the hippocampus, where there is disruption of synaptic plasticity, dendritic atrophy and neuronal death [202].

Gursoy et al. [73] have shown that mice maintained on CR for 8 weeks showed two-fold higher levels of plasma corticosterone levels and also significant decreases in total glucocorticoid receptor levels in the liver, thymus, heart, and testes, as well as higher lipid peroxidation levels in both liver and heart. Taken together these findings suggest that perhaps these changes may counteract the initial protective effects of dietary restriction in senescence. The fact that mild stress may offer some protection against subsequent stressors does not mean that these changes are not biphasic and do not result ultimately in some maladaptive response. Therefore, future research should evaluate the levels of so called neuroprotective markers throughout the life of the animal and not only at a given time point.

### 4.4. Quality of life

Finally, there is the all-important quality-of-life issue. It is obvious from the discussion concerned with the prevalence of obesity that there are numerous individuals with whom a quality-of-life issue may be more important than successful aging. Thus, to reduce caloric intake by a significant percentage in order to extend life and increase protection against the deleterious effects of aging may be less desirable than suffering the consequences later. The question becomes, “What does CR mean to healthy active people?” It is clear that food in humans means a lot more than our means for survival. Food in our species is an entity that is not only related to health but also to culture and socio-economical status. Thus, it may be that a diet high in fruits and vegetables and low in fat is by default a diet low in calories and people following an appropriate diet and exercise regimen are already practicing a form of CR. Therefore, it seems more appropriate to propose a diet rich in fruits and vegetables, which has the added benefits of antioxidant and anti-inflammatory effects, as well as putative direct effects on neuronal signaling and physiological and mental well-being that come from these nutrients. More importantly, it appears that this diet can be initiated at any point in the life span with positive effects, while CR seems to produce its most beneficial effects when begun early in the life span.

At the moment the mechanisms by which either food restriction or fruits and vegetables exert their effects has not been established; however, if we examine the pros and cons of the two interventions, CR seems to carry more risks than does increasing the dietary intake of antioxidants through fruits and vegetables. Therefore, until the issues discussed above concerned with CR in humans are elucidated, we may be able to “have our cake and eat it too” (as long as it is low-fat carrot cake).

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